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Antibacterial Potential of Haemolymph and Aqueous Extraction of Red Velvet Mite, *T. Grandissimum*

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Abstract:

The haemolymph, outer skin and whole body aqueous extracts of the red velvet mite showed a good antibacterial potential against human bacterial pathogens. Of the different bacteria tested antibacterial activity was well expressed against S. *typhi and C. tetani*. The haemolymph showed less activity against (6.7 ± 0.3 mm) *P.aeruginosa*. The whole body extract (200 mg /disc) showed a high inhibitory action against *A. hydrophila* (8.1 ± 0.3 mm) and minimum effect on *S.aureus* (5.6±0.2mm). The extracts of the skin registered a maximum activity against *P. aeruginosa* (7.2 ± 0.1 mm) and minimum activity against *C. tetani* (3.6 ± 0.1). Of the three tissues tested, antibacterial activity was more pronounced in fresh haemolymph. The antibacterial potential in the haemolymph and whole body extracts of Red velvet mite suggests that the mite possess antibacterial compounds. A dose dependent variation was observed in the antibacterial activity. The extracts of haemolymph at a dose level of 200 mg/disc showed the highest inhibitory activity.

Key words: *T.grandissimum, haemolymph, antibacterial activity and pathogens.*

Introduction:

The world health organization estimates that as many as 80% of the world's more then six billion people rely primarily on animal and plant based medicine [1]. In modern societies, zootherapy constitutes an important alternative among many other known therapies practiced worldwide. Wild and domestic animals and their byproducts (e.g. hooves, skin, bones, feathers and tusks) form important ingredients in the preparation of curative, protective and preventive medicine [2].

Red velvet mites are arachnid arthropods inhabiting the subterranean habitats for over several million years and they protect their soft velvet like body and young ones in the soil by some special defense mechanism from soil borne microbes. According to Sharma [3] since insects had evolved over 500 million years ago and flourish in all sorts of habitats, they must be manufacturing a wide assortment of compounds to counter microbes that threaten them. The *Trombidum* species are exposed to a cocktail of nasty bacteria and fungi so that their defense mechanism unleashes all its power against the pathogens. Antibacterial activity of the other subterranean arthropods like termites had been well documented [4-6]. Antimicrobial peptides had been reported from termites [7] and ants [8-9]. Insects had been proved to be very important sources of drugs for modern medicine since they have immunological, analgesic, anti-bacterial, diuretic, anti-rheumatic and anesthetic properties [10]. Beattie *et al.,* [11] had stated that the arthropods that live on close proximity to each other such as wasps, bees, mole crickets, scarablarvae, cicadanymphs, and centipedes are subject to microbial attacks and epidemic diseases. To limit disease activity they incorporate antimicrobial compounds into their nests.

Oudhia had written (Forest information update (FIU) a free weekly email newsletter, No.21, 3 July 2000) that the current rate of red velvet mite, *Trombidium grandissimum* Koch in Chhattisgarh State of India was equivalent to 20 US Dollars / Kilograms, and these mites were used as good sex tonic and its oil is very useful in treating more than 50 common diseases. But there is no scientific study on the antibacterial activity of the extracts of the red velvet mites. Hence in the present study attempt has been carried out to find out the antibacterial activities of the extracts of the whole mites, skin and fresh haemolymph.

Table 1: Antibacterial activity of whole mite (*T.grandissimum*) extracts against pathogenic bacteria in Disc plate method.

Organisms	Name	Zone of inhibition (mm) (± S.D) Concentration of whole sample (mg/disc)			Standard antibiotic Ciprofloxacin
		100	150	200	(40µg/ml)
Gram Positive Organism	*S. aureus*	3.3 ±0.1	4.1 ± 0.3	5.6 ± 0.2	14.3
	S. pneuomoniae	4.1 ± 0.2	4.6 ± 0.2	6.2 ± 0.3	16.5
	C. diphtheria	4.1 ± 0.1	5.1 ± 0.1	6.3 ± 0.2	16.4
	B. cereus	4.2 ± 0.3	5.1 ± 0.1	7.2 ± 0.1	18.4
Gram Negative Organism	*C. tetani*	4.1 ± 0.2	5.6 ± 0.2	7.1 ± 0.3	19.3
	E. coli	4.0 ± 0.3	5.4 ± 0.3	6.3 ± 0.2	16.6 19.4
	S. typhi	4.5 ± 0.1	5.2 ± 0.1	6.4 ± 0.1	
	A. hydrophila	5.4 ± 0.1	5.1± 0.1	8.1 ± 0.3	26.3
	K. pneuomoniae	4.1 ± 0.3	5.4 ± 0.3	7.7 ± 0.2	25.4
	P. aerugenosa	4.2 ± 0.2	5.1 ± 0.2	7.2 ± 0.2	24.1

Table 2: Antibacterial activity of skin extracts of mites, *T.grandissimum* against pathogenic bacteria in Disc plate method.

Organisms	Name	Zone of inhibition (mm) (± S.D) Concentration of skin extract(mg/disc)			Standard antibiotics Ciprofloxacin
		100	150	200	40µg/ml
Gram Positive Organism	*S. aureus*	3.2 ±0.2	4.2 ± 0.2	5.1 ± 0.2	14.3
	S. pneuomoniae	3.4± 0.1	4.2 ± 0.1	5.4 ± 0.1	16.5
	C. diptheriae	3.2 ± 0.3	4.3 ± 0.2	6.6 ± 0.3	16.4
	B. cereus	3.2 ± 0.2	3.7 ± 0.3	4.2 ± 0.2	18.4
Gram Negative Organism	*C. tetani*	3.3 ± 0.1	3.2 ± 0.1	3.6 ± 0.1	19.3
	E. coli	4.2 ± 0.2	4.2 ± 0.2	4.6 ± 0.3	16.6
	S. typhi	4.4 ± 0.1	5.7 ± 0.3	6.2 ± 0.2	19.4
	A. hydrophila	3.4 ± 0.1	5.5 ± 0.1	7.6 ± 0.3	26.3
	K. pneuomoniae	2.6 ± 0.2	3.7 ± 0.2	4.6 ± 0.2	25.4
	P. aerugenosa	3.4 ± 0.2	5.2 ± 0.1	7.2 ± 0.1	24.1

Material and Methods:

Antibacterial assay: The human bacterial pathogens such as *Staphylococcus aureus, Salmonella pneumonia, Clostridium diphtheria, Bacillus cereus, Clostridium tetani, Escherichia coli, Salmonella typhi, Aeromonas hydrophila, Klebsiella pneumonia* and *Pseudomonas aerugenosa* were selected for antibacterial screening. Ten microlitre of the bacterial broth culture was aseptically transferred to the air dried sterile agar plates and spread the culture uniformly with the help of a sterilized spreader made up of glass rod.

The extracts of outer skin, whole body and fresh haemolymph were subjected to pilot study. A pilot screening of the extracts were carried out by impregnating a 6 mm sterile Whatmann number.1 filter paper discs. The disc was loaded with the extracts to give a final load of 100,150 and 200 mg /disc. The discs were allowed to dry completely and

Table 3: Antibacterial activity of fresh haemolymph of mites, *T.grandissimum* against pathogenic bacteria in Disc plate method.

Organisms	Name	Zone of inhibition (mm) (± S.D) Concentration of hemolymph (mg/disc)			Standard antibiotics Ciprofloxacin
		100	150	200	40µg/ml
Gram Positive Organism	*S. aureus*	4.2 ±0.2	4.6 ± 0.2	7.2 ± 0.3	14.3
	S. pneuomoniae	4.6 ± 0.2	5.6 ± 0.3	8.1 ± 0.2	16.5
	C. diptheriae	4.2 ± 0.3	5.7 ± 0.1	7.2 ± 0.3	16.4
	B. cereus	3.6 ± 0.2	5.2 ± 0.2	8.3 ± 0.2	18.4
Gram Negative Organism	*C. tetani*	5.2 ± 0.1	6.6 ± 0.1	8.6 ± 0.1	19.3
	E. coli	5.2 ± 0.1	6.2 ± 0.2	7.6 ± 0.2	16.6
	S. typhi	6.2 ± 0.2	6.6 ± 0.1	8.4 ± 0.2	19.4
	A. hydrophila	3.3 ± 0.1	6.2 ± 0.3	8.3 ± 0.3	26.3
	K. pneuomoniae	4.2 ± 0.3	5.2 ± 0.2	7.4 ± 0.1	25.4
	P. aerugenosa	3.2 ± 0.2	4.2 ± 0.1	6.7 ± 0.3	24.1

after the aqueous solvent was evaporated, the discs were placed on the Petri plate previously seeded with the respective bacterial strains. Three replicates were used for each treatment. Control discs were kept without any extracts but soaked in respective microlitre of aqueous solvent and dried plates were then kept at 37^0 C in an incubator for 24hrs. The inhibition – zone width (distance from the edge of the paper disc to the outer edge of the inhibition zone) was measured to the nearest mm, at 24hrs by using Hi-Media antibiotic zone scale and expressed in standard deviation of mean (± SE).

Results and Discussion:

Antibacterial activity: Results on the antibacterial activity of various extracts of red velvet mite are presented in the Tables 1 –3. The haemolymph, outer skin and whole body extracts of the red velvet mite showed a good antibacterial activity. Of the three tissues tested, antibacterial activity was more pronounced in fresh haemolymph. Next to haemolymph, the whole body extracts showed a good antibacterial activity. Of the different bacteria tested antibacterial activity was well expressed against S. *typhi and C. tetani*. The haemolymph showed less activity against (6.7 ± 0.3 mm) *P.aeruginosa*. The whole body extract (200 mg /disc) showed a high inhibitory action against *A. hydrophila* (8.1 ± 0.3 mm) and minimum effect on *S.aureus* (5.6±0.2mm). The extracts of the skin registered a maximum activity against *P. aeruginosa* (7.2 ± 0.1 mm) and minimum activity against *C. tetani* (3.6 ± 0.1)

It is widely accepted that plants, animals and their by-products used as a source of folk or traditional medicines indicate the presence of a biologically active constituent(s) in them. A significant portion of the currently available non-synthetic and/or semi-synthetic pharmaceuticals in clinical use is comprised of drugs derived from plants, animal, microbial, and mineral products [12]. However many animals have been methodically tested by pharmaceutical companies as sources of drugs to the modern medical science [13]. Approximately 109 animals and their 270 uses are reported in folk medicine in different part of India. The number of animals reported for medicinal purposes in different parts of India is enough to feel a need to discuss on the use of animals and their products, as medicines. In order to stress how important animals were, are and can be as sources of pharmacological substances and discussion

on the use of the animals and their products, as medicines in conservation biology and sustainable use. We have concluded red velvet mites also one of the important zoo therapeutic agent for modern medicines. The antibacterial potential in the haemolymph and whole body extracts of Red velvet mite suggests that the mite possess antibacterial compounds and this has to be explored in future.

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